# The Degenerate (Apyrene) Sperm-formation of Moths as an Index to the Inter-relationship of the Various Bodies of the Spermatozoon.

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With Plate 26.

## INTRODUCTORY.

IN late years much attention has been given to certain atypical spermatozoa in some insects and molluscs. On the latter especially a good many papers have appeared, and there has been much conjecture as to the function (if any) of these curious bodies.

In some molluscs (Strombus, Reinke (1)) the atypical sperm is a most remarkable object, and many times larger than the typical sperm.

The atypical spermatozoa have been called " apyrene " and " oligopyrene " according as to whether they were thought to contain no, or little, nuclear matter.

That in insects, and this applies to the Lepidoptera, an atypic sperm so remarkably different from the typic as is the case in many molluscs, is ever found is extremely doubtful. But it is noteworthy that in such widely different forms as Lepidoptera and Prosobranch Mollusca, an analogous dimorphism should occur. We are tempted to inquire what in common have the conditions of these forms, and why, if these apyrene spermatozoa serve a special purpose, should nearly identical conditions arise under such widely different

environments. In the Holometabola, we get remarkably
varying stages of life history, for which assuredly we would
look in vain in Murex or Tritonium, and, while admitting
that in many Prosobranchs the case is so remarkable as to
permit speculation and theory, I feel that in the Lepidoptera
we cannot attach any importance to the atypical spermatozoa
other than that of a degeneration product.

The special purpose of this paper is an inquiry into these
degeneration products in moths and butterflies, but the apyrene
sperm of the mollusc has formed the subject of a note.

I have to thank Dr. Goodrich for his kind interest and
criticism. This work was carried out in the Department of
Physiology, and in this connection my best thanks are due to
Prof. Sherrington.

## Previous Work.

Meves (2) alone has given a good account of these atypic
spermatozoa of moths. But the atypic sperms described by
him (Pygæra) are not the only kind of apyrene formation of
moths, and it has been the purpose of this paper to point out
that it would be a mistake to think that apyrenes arose exclu-
sively from the time of formation of karyomerites as seems to
occur in some molluscs (1).

Quite recently H. Federley [1] (3) has reported upon the
spermatogenesis of Pygæra anachoreta, P. civitula,
and P. pigra, and has been able to describe as much as his
Carnoy technique would allow. Needless to say this fixative
has spoilt all cytoplasmic details except the amphiaster, and
his results from the point of view of this present paper have
been less successful than those of Meves. Federley has
given a completely satisfactory account of the apyrene
maturation divisions in so far as the behaviour of the chromo-
somes is concerned. This observer also gives an account
of the chromosomes of the hybrids of the above species of
Pygæra, which he has worked out well.

[1] For the loan of this paper I am indebted to Dr. J. W. Heslop
Harrison.

Meves worked upon Pygæra bucephala, and has given a correct account of every stage, except that he has failed to note the behaviour of mitochondria in spireme formation, of the acrosome and of the centrosome. These important stages are considered in the present paper, and the material used has proved more favourable than Pygæra.

In the Metazoa most of the latest work has shown that the spermatid about to become a spermatozoon contains the following bodies : Nucleus, centrosome (or centrosomes), mitochondria, acroblast, and one or two basophil or siderophil bodies besides. The latter, which might have some special significance, are not dealt with here.

The special purpose of this paper is to attempt to analyse the inter-relationship of the first four bodies in the spermatid. We know that in spermiogenesis, and by this term I mean the stages from spermatid onwards, the nucleus, centrosome, acroblast, and mitochondria undergo a number of definite movements and changes which finally culminate in the pro- duction of that remarkable cell, the spermatozoon. In moths, and may be in many other forms, degenerate stages of spermatogenesis occur, and it has been by observing these stages in the former that I have attempted to come to some conclusion concerning the various functions and the various influences which together go to produce the sperm. If, in some cases, my conclusions be not clear, it is because I have found great difficulty in identifying the special cell element which might be a centre of influence for another cell body, but it will be seen that the evidence provided permits one to make tentative statements.

The technique employed was similar to that used in my previous paper (4). Smerinthus populi and Pieris brassicæ were most satisfactory forms upon which to work ; in addition Pygæra bucephala and Porthesia similis were examined. The character of the apyrene or degenerate sperm-formation was found to be somewhat different in different species. Thus in my Pierid material almost, if not invariably, degeneration, or the occurrences leading to the

formation of an apyrene sperm, began after the establishment of the spermatid nucleus. In Pygæra the second maturation chromosomes failed to fuse at all in most cases, while, on the other hand, a transition between Pygæra and Pieris was provided by Smerinthus where several nuclei became formed in the spermatid from a number of successfully fused chromosomes.

### The Nucleus in the Degenerate Stages.

In Pl. 26, fig. 4, is drawn a fairly typical second maturation division of the degenerate type which will lead on to the abnormal sperm. The two cells are almost constricted from each other, but the chromosomes ($C.H.$) are straggling, as if the centrosome and astral rays lacked the energy to finish anaphase and telophase. The mitochondria are normally constricting. The acroblasts are not quite normal, but are in the usual position in which they are found. The micromitosomata are normal. Now this figure is typical of many maturation divisions; it shows what most other degenerate stages do—that the disintegratory process seems traceable at first to the chromatin. It is almost, if not quite invariably the nucleus which, in the abnormal growing spermatocyte, shows signs of degeneration, and it is the nucleus in other stages (Pl. 26, figs. 6, 8, 10, etc.) which is the first cell element to fail. Inspection of Pl. 26, fig. 4, might lead one to believe that the centrosome is at fault, but this seems negatived when one remembers that in later stages the centrosome still has the energy to keep its position at the head of the cell, to undergo some normal changes, to form the axial filament as usual, and finally to enter into its normal relations with the mitochondrial spireme. This, at least, points to the fact that one must be careful in imputing this abnormal telophase to the amphiastral rays. In the telophase of normal mitosis the chromosomes fuse, and afterwards a new nucleus is formed. In these abnormal stages this process rarely takes place in the usual manner. The chromosomes either do not

fuse at all, or only a limited number fuse. These latter, as well as the isolated single chromosomes, endeavour to form new nuclei, and one gets the sort of cell drawn in Pl. 26, figs. 1, 2, and 6. Pl. 26, fig. 2, is quite typical. There were in the field seven abnormal nuclei, none of which had succeeded in forming a proper reticulum. In Pl. 26, fig. 6, there were some five nuclei, the two bottom ones of which were normal in appearance. In Pl. 26, fig. 1, the truth of these remarks is also illustrated ; some nuclei were apparently normal, though smaller ; others were abnormal. Degeneration of the nucleus takes place at every stage of spermiogenesis. Even when the nucleus of the spermatid is apparently normally formed, it may in some other way show that it is not properly functional. Pl. 26, figs. 3, 7, 8, 10, and 11, show degenerate stages in which the nucleus, though seemingly normal, fails to become attached to the centrosome, and drifts down the growing sperm. The centrosome keeps its position. In Pl. 26, fig. 13, the several nuclei formed from the number of chromosomes which failed to join up all together have undergone the usual staining change which takes place at this period, but only one nucleus has managed to keep its position at the head of the cell. In Pl. 26, fig. 12, the very darkly staining nucleus has drifted down the sperm, and has attached to it a small acrosomic granule ($G.$), which does not seem to be quite normal. In Pl. 26, fig. 9, the nuclei are normally reconstituted, but, judged from the progress of the acrosome, are late in reaching this stage. All the nuclei in this nest were unable to keep their positions at the head of the cells. The section is somewhat oblique.

With regard to the formation of the nuclei from single or fused chromosomes, every conceivable stage can be found, especially in Smerinthus and Pygæra, where failure to form a proper spermatid nucleus is common ; this also applies to Spilosoma, but in Pieris brassicæ failure generally comes at the stage when the spermatid nucleus should adhere to the centrosome (Pl. 26, figs. 10 and 11, etc.). Pl. 26, fig. 13, shows what often occurs in Smerinthus and Pygæra.

A very important point to notice is that degeneration of the nucleus may occur at any stage, but in some species it tends to take place at certain special stages. It should be mentioned that in different bundles the individual spermatids all tend to reach the same stage of development, degeneration appearing at one and the same time throughout. Some spermatozoa get further than others in development, and apparent arrival at the last step of sperm-formation does not necessarily mean that the canker of degeneration may not appear later when the sperm has entered the egg. Thus development might be initiated by such spermatozoa without the male nucleus being able to fuse. But I do not believe that the so-called "apyrene" sperm could enter the egg, though the suggested effect of an "apyrene" sperm might be got by degeneration overtaking a normally constituted spermatozoon at some stage in fertilisation. If a spermatozoon entered an egg, if its nucleus began to lag, as it often does in spermiogenesis, and if the centrosome acted normally, one might possibly get development initiated, and there is no doubt that every sperm from the same bundle would act likewise. There is nothing illogical in the thought that if degeneration can overtake the sperm at any stage of spermatogenesis, it could also overtake it at any stage in fertilisation.

In Pl. 26, fig. 14, I have drawn a degeneration stage in Pygæra bucephala. The nuclei have shrunken into darkly staining lumps, as is usually the case when any nucleus degenerates completely. Even after this stage a partial elongation of the cell may take place.

## The Mitochondria.

In my previous paper I stated that it was impossible to make out the relationship of the macromitosomal spireme (nebenkern) and the centrosome. By means of degenerate stages such as those drawn in Pl. 26, figs. 3 and 11, it is clear that the macromitosome consists of a spireme, the front free end of which is attached to the centrosome. The abnormal

sagging of the macromitosome has caused the method of fixation to the centrosome to be revealed, though how the other end of the spireme is attached I do not know. In the normal spermatid the stage drawn in Pl. 26, fig. 6, gradually passes to the stage of a tangled spireme (Pl. 26, fig. 2, etc.). Now when the spermatid begins to elongate, the attached front end of the spireme remains in its position, and the rest of the mitochondrial matter becomes "let out" or "played out" as the elongation takes place. This process has taken place normally from $C.$ to $Y.$ in Pl. 26, fig. 12, but somehow or other the spireme subsequently became apparently refractory $(M.)$, and was left in this position, while the lower part $(Z.)$ made an endeavour to form normally as the elongation went on. In Pl. 26, fig. 5, another sort of abnormal formation is shown : here the macromitosome, instead of pulling out in a smooth, even manner, came out in lumps $(M.^1–M.^6)$. In Pl. 26, fig. 3, the spireme is loosing its position en masse. It seems that the retention of the spireme in its place, or at least its completely normal elongation, rarely takes place after the failure of the nucleus to act properly. This may be due to the general abnormal condition in the cell.

### The Acroblast and Acrosome.

This body can almost invariably be found in the early spermatid, and in those cases where its presence cannot be ascertained until sperm-formation is well advanced, special refinements of technique may succeed in demonstrating it early. In the case of the snail I have experienced unusual difficulty in studying this body. I have found that bichromate of potash is extremely favourable for fixing the acrosome, and osmic-bichromate fixatives are indicated in this section of spermatogenesis studies. For instance, by smearing the ovotestis of Helix on a slide, momentarily fixing in osmic acid fumes, and then, after allowing the film to dry partially, dipping into a 2 to 5 per cent. bichromate of potash solution, an extremely intense stain of the acrosome is got if the slide is soaked long enough in the bichromate solution. My work

has not yet been carried out far enough to allow me to attack with any complete surety the view that the acrosome is derived either partly or wholly from the sphere or archoplasm, but I confess that I consider this suggestion improbable ; not only because I cannot allow that the observers who espouse this view have shown clearly that such is the case, but also because the fixatives most favourable, or at least favourable enough to demonstrate archoplasm, at the same time uniformly unfavourable to acroblastic material. One result of this study of the atypical spermatozoon has been the confirmation of a suggestion advanced by me in a previous paper (**4**), that the acroblasts in later stages are influenced by the nuclear matter alone.

Figures have already been given to show that in the maturation divisions the acroblasts of Lepidoptera are orientated in a remarkable and special manner in relation to the chromatin, and it has been shown that this relationship is rarely departed from ; a review of some of the best work on the plasmatic structures in other orders leads me to conclude that, just as in moths and butterflies, the acrosomic material is definitely and undoubtedly subservient to the nucleus at almost all stages. The acroblast (or acroblasts) is almost always found to lie in the cytoplasm in the neighbourhood of the nucleus, and sooner or later takes up its position upon the surface of the latter. Now, a very important question to solve was whether this special orientation of the acroblast upon the surface of the nucleus could be brought about by the instrumentality of any body other than the latter.

Meves, in his work on the " apyrene " spermatozoa of moths, completely overlooked the acroblasts, and my own work on the same species as he used leads me to believe that Pygæra bucephala [1] is not good material for this study. Pierids and Smerinthids are very favourable. It is known that in the so-called " apyrene " or atypic spermatozoon the nuclear matter fails to act normally and gradually becomes carried down

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[1] I have since ascertained that in P. bucephala the acroblasts are difficult to discover because they so closely resemble mitochondria in size and shape.

the body of the lengthening sperm. The chromatin is thus removed from what is the head end of the spermatozoon; now, in the normal sperm the head end is formed by the acrosome. In the atypic sperm the centrosome forms the head end, and a very remarkable fact is that the acroblasts in the Lepidopterous apyrene sperm follow the chromatin down in its path along the lengthening spermatozoon. In Pl. 26, figs. 3, 5, 7, 8, 11, 12, and 13, this is shown. If the centrosome needs to be fairly near the body it influences, we would be justified in assuming that the central corpuscle does not influence the acroblasts, for they lie a considerable distance from this body. Reference to my previous paper will show that the acroblasts of moths keep close to the spermatid nucleus, and after approaching one side of the latter, fuse. The fused or partially united acroblasts secrete a granule which lengthens to form the acrosome. These events happen synchronously with the special changes in the nucleus which leads to the formation of the elongated, apparently solid, sperm nucleus; thus by the time the nucleus is elongated, the acrosome is also elongated and pointed. It has already been shown that the chromosomes in failing normally to come together to form the spermatid nucleus, often tend to form several nuclei of small and varying size. The nuclei fail to retain their position at the sperm head and drift downwards. The acroblasts almost invariably follow at the same time, and also tend to apply themselves upon the chromatic matter as they do in normal spermatogenesis. We then come to realise that though the chromosomes lack that power which normally allows them to reunite to form the spermatid nucleus, nevertheless they still are able to influence the acroblasts. Moreover, one often discovers a spermatid in which one small nucleus, probably formed from one chromosome, can be seen shepherding several acroblasts which are stuck on its surface. In these cases I do not think that a normal acrosome is found in later stages. That this attendance of the acroblasts upon the partially regenerated nuclei is not accidental, and caused by the fact that any bodies tend to become cast down the

length of the sperm together, is shown by the fact that one cannot find many straggling acroblasts (see Pl. 26, fig. 10), and that the latter are able to form an acrosome upon the surface of the larger nuclei in later stages of degeneration. Even in normal stages the steps which lead to the formation of the acrosome from the acroblasts are never exactly synchronous in a sperm nest, but they rarely vary beyond certain limits. In the spermatocyte and spermatid the acroblasts are semilunar in shape in normal stages, and this shape gives way to a vesicular one. Now the acroblasts in abnormal spermatids quite often show a tendency to shrink and become darkly staining at a stage when in normal cells the acrosomic granule is being secreted, and since these darker acroblasts are often found removed from the nuclei, it might be possible to assume that the changes leading to the formation of the acrosome are not wholly dependent upon the nucleus. This seems to be supported by the fact that the acroblasts, when not in the immediate neighbourhood of the nucleus, may be found even in normal stages of different degrees of metamorphosis towards acrosome. Another significant fact is supplied by Pl. 26, fig. 9, which shows a part of an abnormal spermatozoon-nest in which the nuclei, though normally reconstituted after the second maturation division, have failed to keep their position in the cell (see also Pl. 26, figs. 3, 5, 7, 8, 10, 11, and 12), but on drifting down have been followed by the acroblasts, which have formed complete acrosomes. The latter (A.) are normal in every degree, but the nucleus has failed to develop synchronously, and the curious condition is got of one element racing another in metamorphosis. The fact to be noted at present is that the acrosome can be formed without the synchronous steps in the nucleus. The sharp acrosome in Pl. 26, fig. 9, belongs to a stage when the nucleus is also elongated and when it has regained its affinity for nuclear stains. If the metamorphosis of the acroblasts depended absolutely upon the nucleus one would get these bodies still vesicular and just about to apply themselves to the nucleus, but instead they are now fully formed. I would interpret Pl. 26, fig. 9, as follows:

The nucleus lacked the requisite force to place itself at the head of the sperm, but retained its relationship to the acroblasts, which fused, secreted the acrosome, and gave rise to a normal structure. But that missing power, whatever it may be, still was wanting in the nucleus, and evinced itself by causing a lagging in the proper formation of the elongate sperm nucleus. This lagging, be it marked, did not affect the acrosome, which became normally formed, and so the condition in Pl. 26, fig. 9, was reached. In Pl. 26, fig. 13, each of the four nuclei is attended by a small round granule (*G.*), which is probably an acrosome, but in the cell normal acroblasts (*A.*) were still present. In Pl. 26, fig. 12, the nucleus had a granule applied upon its surface (*G.*). It is probable that if this body arises from an acroblast it is abnormal, for I have not found like stages in normal nests; in normal stages such a granule would be surrounded by semilunar acroblastic ring (see figures in previous paper). The question of the attachment of the acrosome upon the nucleus may now be dealt with ; in the snail I find that the acrosome is embedded in the nucleus in a wedge-like manner. In some other forms, as, for instance, in the rat (after Duesberg (**5**)), the acrosome is plastered upon one side of the nucleus. In some other Mammalia the acrosome seems to be fixed straight across the square head of the nucleus. In fact, almost every conceivable manner of joining between these two elements can be found.

My study of Lepidopterous spermatozoa leads me to believe that the acrosome is sensitive to some influence which orientates it. Pl. 26, fig. 9, shows that each acrosome, though it has no normal nucleus to fix upon, is sensibly orientated towards the sertoli-cell or upper end of the sperm-nest. It is quite probable that the front end of this acrosome in Pl. 26, fig. 9, represents what would be the front end of the acrosome in the normal sperm. Now the acrosomes in Pl. 26, fig. 9, are not all resting upon the nucleus, and the same applies to Pl. 26, fig. 15, in which the arrow denotes the head or front end of the nest. In

Pl. 26, fig. 13, the granule (*G.*) if really an acrosome
is not orientated towards the front of the nest, but
in the greater number of cases in all abnormal stages
the acrosome keeps towards the front end of the cell.
What cell element causes this sensible orientation? Is it
centrosome or nucleus? That it is not the former might
be concluded from the history of the normal sperm where
the centrosome is at the back of the nucleus, and in a
position which one might be justified in presuming, unable
to affect the acrosome. The facts of the influence of the
acroblasts upon the nucleus, and of their very apparent
inter-relationship leads me to believe that the nucleus might
have something to do with this orientation of the acrosome
in the direction of the head end, though more probably the
whole question should be considered in relation with the
growth of all the elongating spermatozoa in one direction,
which is a fact which has already attracted attention. In
the worm (Lumbricus) the spermatozoa grow outwards in
a beautifully regular manner to form a ball, and here there
is no sperm nest-wall. In several abnormal bundles of moth
spermatozoa I have found that this growing outwards in
a common direction by all spermatozoa is not completely
perfect. In the testis the bundles of normal spermatozoa are
cut across in all directions showing that this growing out
of all tails in one way is a matter concerning individual
bundles. I have not examined the part played by the sertoli
or nurse-cells with sufficient completeness to give even a
tentative statement, but I believe that this cell may be
intimately concerned in the orientation of sperm and sperm
elements.

The partial isolation of the acrosome from the nucleus in
abnormal stages shows that the element which causes the
adherence of one body upon the other is here lacking or
inefficient. Just as the nucleus is often unable to adhere
to the head centrosome, or vice versâ, so the acrosome
sometimes may lie quite near, quite completely formed, but
lacking the power to become adherent. The manner by

which the fusion of acrosome and nucleus is brought about
is difficult to understand. Whether it is caused by some
intermediate body, or by mechanical means can only be
ascertained with a very small degree of probability, but my
work on the snail and other forms leads me to believe that
even if adherence is brought about by some intermediate
substance, the efficiency of this latter substance is improved
by either the fixation of the acrosome upon the nucleus,
like the ferrule of a walking-stick, or by the wedge-like
embedding of the acrosome into a cavity in front of the
nucleus (Helix). One is led to conclude that in the moth
it is only after or when the nucleus of the sperm has become
elongated that complete fusion with the acrosome becomes
established. Pl. 26, fig. 9 seems to show that this fusion
depends on the nucleus and not on the acrosome.

### The Centrosome.

In the normal stages of spermatogenesis, the centrosome
from which the axial filament grows, becomes applied to
one side of the nucleus and in most forms becomes modified
in some special manner. It may become flattened upon the
nucleus, or squared off as in some mammals. In some cases
it may not appear to have actual physical continuity with
the nucleus. In Lepidoptera the centrosome becomes
cushion-shaped upon the nucleus and as the latter elongates,
the central corpuscle also becomes drawn out slightly. In
those cases where the nucleus fails to become fixed upon
the centrosome, the latter eventually forms the head of the
sperm (Pl. 26, figs. 5 and 12.) As far as I can ascertain from
my sections the centrosome loses its spherical shape (see Pl. 26,
fig. 2) and becomes elongate as in Pl. 26, figs. 7 and 8. Meves'
figures of abnormal stages support this view. One concludes
from this that the centrosome per se, goes on with its
slight changes even though the nucleus has drifted aside.
This is in accordance with what happens in other cell
elements, which appear to go on developing with at least
partial independence. It is well to notice that after the

failure of the nucleus in the metamorphosis of the cell elements of the spermatid the centrosome invariably retains all the energy necessary to undergo its normal changes. One is forced to believe that the central corpuscle of all the plasmatic elements is the most resistant to those influences which cause abnormality, and this should be borne in mind in the discussions relative to any possible part played by "apyrene" spermatozoa.

## DISCUSSION.

The main fact which we must keep before us in a review of the evidence provided by these abnormal stages is that it would be unwise to conclude, because one body is somewhat far removed from another, that mutual influence is so precluded. Because the centrosome lies at the head of the atypical sperm, and the abnormal nuclei and their acroblasts far down, we are not justified in at once dismissing any possibility of the one affecting the other. Nevertheless I feel that we can conclude a good deal from such evidence as we have at hand, though some suggestions must only be very tentative. I have already shown that though the nuclear matter is abnormally affected, still the acrosome may be formed, and in the same way a normal macromitosome may be developed from the granules. The tail of the sperm may be normally formed by a thinning out and elongating of the macromitosome, and otherwise, excepting the chromatin, the cell elements may be quite normal. I believe that this shows that we are concerned with two special occurrences in spermatogenesis : Firstly, the formation of the acrosome near the nucleus, and the evident inter-relationship of these bodies ; and secondly, the growth of the tail filament, and the special grouping of, or formation of, an envelope from the mitochondrial granules. These two separate occurrences appear to be able to take place with independence of each other, and I think the two apparent free forces in the spermatid are respectively lodged in the nucleus and in the centrosome

The final linking up of all the occurrences in the sperm-cell and their proper consummation depends on the correct attraction between centrosome and nucleus at the time when the various cell elements become formed up in line preparatory to the elongation of the spermatid.

The growth of the sperm tail in length depends, I believe, absolutely on the head centrosome. Now the centrosome also is intimately connected with the macromitosome (nebenkern of some authors), at least in the later stages, when the elongation is taking place, and in the moths the centrosome is certainly connected with one end of the mitochondrial spireme. Yet we know quite well that in cellular division the astral rays are certainly not connected with either single mitochondria or groups of mitochondria. It therefore seems that a new relationship betwixt centrosome and mitochondria is brought about in later stages of sperm-formation. The curious and definite groupings of mitochondria around the filament in such a case as Enteroxenos might possibly be otherwise accounted for, but I believe the centrosome to be mainly responsible. The acroblast also offers curiously contradictory evidence; it undoubtedly (in moths, at least) becomes definitely oriented towards the nucleus in several stages of spermatogenesis, and yet in mitosis it keeps apparently completely within the zone of the astral rays, near the centrosomes. From the maturation divisions and subsequent spermiogenesis I conclude that the following inter-relationships could be demonstrated between—

(1) Nucleus and acroblasts.

(2) Acroblasts and centrosomes (in cell division alone).

(3) Mitochondria and centrosome only in later stages (after spireme formation).

(4) Centrosome and nucleus (not chromosomes) only in later stages, when both bodies become adherent one to the other. (I do not here refer to division of the cell.)

The question of the relationship of mitochondria to other cell elements in cell division I believe to be settled by some stages, especially in the prophases, where in moths the seed-

like mitochondria tend to become fused fan-wise from the zone of influence of the centrosome. It is nevertheless quite true that the mitochondria, though often so affected, almost invariably in later stages keep well outside the amphiaster. This seems to show, as well as much other such evidence does, that the mitochondrial granules are not absolutely equally, but only approximately equally divided. It is quite certain that the mitochondria are never divided in such a correct manner as the chromosomes.[1] Some stages given by me elsewhere show that this approximately even division of the mitochondria may be departed from, resulting in a very perceptibly uneven distribution. Even if the suggestion of two special capital centres of force in the spermatid be not completely substantiated, it is quite certain that spermio genesis is the sum result of the separate workings of a number of forces, and these latter, when not working in unison, produce abnormal spermatozoa. The curious spermatogenesis of some hybrids might be traced to a want of unison in the arrangement of the elements provided by both sides. It has been suggested that the growth of the sperm-tail depends upon the head centrosome. Whether this applies to the alteration of shape in the nucleus and acrosome is, indeed, difficult to say. The nucleus, after losing its place at the sperm-head, is still able to become elongate, and the same remark applies to the acrosome.

The main conclusion is that even though certain cell elements in spermatogenesis become degenerate, others may be able to go further on with apparent semi-independence, and may even become normally formed.

### The Degenerate Spermatozoon as a Probable Special Sex Determinant.

Hertwig (6) suggested that the "apyrene" spermatozoon might by fertilising an egg produce offspring of a different sex from an egg fertilised by a "eupyrene" sperm. Other

[1] See, however, E. B. Wilson on Centrurus, 'Nat. Acad. Sciences,' vol. 2, June, 1916.

authors have already pointed out that it has not satisfactorily
been shown that the "apyrene" is able to enter the egg.
The view taken in this paper is that the atypic sperm, in
moths at least, has no special significance beyond the fact
that it originates from a state of degeneration. I have shown
in the figures in this article that almost every conceivable
stage in degeneration from spermatocyte onwards can be
found in lepidopterous spermatogenesis, and even if there
appears to be a special, true "apyrene" sperm, which I
doubt, this is because it is at one special stage that degenera-
tion is most prone to appear. I have already pointed out (4)
that degeneration cannot be due to a starved condition of the
larval or pupal moth or butterfly, but exactly why degenera-
tion should appear at all I cannot say. Nevertheless I
consider it certain that the appearance of "apyrene and
oligopyrene" spermatozoa is directly traceable to the same
forces which cause a whole nest of primary or secondary
spermatogonia to undergo degeneration. Even if subsequent
research should show that fertilisation by an atypic sperm is
possible, this cannot show that the latter has a special sig-
nificance from the sex point of view, though it would intro-
duce an element of very strong probability. I have already
remarked, in the section dealing with the nucleus, that a
sperm might be overtaken by degeneration after it had
entered the egg. This matter will easily be settled by a
cytological examination of enough fertilisation stages, together
with a comparison between the expected and the true sex
ratios in breeding experiments.

### The "Apyrene" Spermatozoon of the Moths and of the Molluscs.

Among later workers on the atypical spermatogenesis of
Mollusca there is a clear consensus of opinion that the
"apyrene" sperm could not fertilise the egg, and this seems
completely substantiated by Reinke's (1) inability to find any-
thing but eupyrenes in the receptaculum seminis of Strombus.

Goldschmidt (7) advances the view that the "apyrene"

spermatozoa of moths are reaction products, probably caused by the changes in the chemical properties of the hæmolymph during metamorphosis. He says : " In the case of Samia it is easy to observe, without going into chemical details, that the blood in old pupæ, which produce the atypical spermatozoa, has very different properties from those in the young." It must be admitted that there may probably be some truth in this plausible explanation ; but, while believing that " apyrene " spermatozoa may be caused by some subtle alteration in the hæmolymph, it is well at present to accept Goldschmidt's explanation with caution, and for the following reasons :

(1) In different larvæ and pupæ of moths and butterflies, though individuals may be sub-equal in size, the development of the spermatozoa may have reached much later stages in one than in another example. No rigid synchronism here holds good.

(2) In some forms like Spilosoma, all sperm-formation is finished by the time the larva is full grown. In other forms which appear at the same time and have the same sort of life cycle, sperm-formation takes place mainly in pupæ.

(3) Apyrene spermatozoa may be the first kind to reach development in the testes. Eupyrenes may only appear much later.

(4) Apyrenes and eupyrenes may be found developing side by side at what appears to be the same stage.

(5) Apyrenes appear in Molluscs where the suggestion of altered conditions of a blood fluid would not seem to apply, and where no metamorphoses takes place.

With regard to the statement in paragraph three, the following is my experiment : A number of Pygæra bucephala larvæ pupated in September. The testes then contained only spermatogonia and spermatocytes not quite grown to their full size. The pupæ were kept in the warm laboratory, and near the end of October it was found that the testes contained several bundles of spermatozoa and spermatids, all of which were abnormal or apyrene. About Christmas the

testes were found full of bundles of both sorts, apyrene and the normal or eupyrene metamorphosing side by side. According to Goldschmidt, I suppose the explanation of this would be that the hæmolymph in early stages favoured the formation of apyrenes, but that later in histolysis the fluid of the body became adjusted suitably for eupyrenes. Whether this explanation can be held good might be proven directly by injecting some chemical substances into the pupa, and by altering the hæmolymph provide an easy basis for comparison with what occurs in the control pupæ.

In molluscs it seems that in such a case as Paludina, atypic spermatozoa arise from ordinary spermatids, but in Strombus Reinke traces the apyrenes from special cells not to be identified as normal spermatogonia. How could Goldschmidt's explanation apply to either case in molluscs? I believe the example of the moth testis, where both sorts of spermatogenesis goes on side by side is distinctly comparable to that of the molluscs. Why does not the chemical unsuitability of the hæmolymph apply alike to all spermatid bundles?

In connection with these cases of remarkable dimorphism in spermatozoa, due to degeneration, the noteworthy case of Nerilla should be mentioned (Goodrich (8)). In this archiannelid the male has three genital segments : the first of these, the seventh, alone produces normal spermatozoa, but the eighth and ninth give rise to curious cells with granules ; the exact category under which these cells should be placed, whether spermatogonia, spermatocytes, etc., and the manner in which the granules appear have not yet been elucidated, but there can be little doubt that the conditions leading to abortive spermatozoa in segments eight and nine are to be identified with the same forces which cause the apyrene sperm of moths to appear.

Reinke (1) has some interesting suggestions to make regarding the apyrene spermatozoa of Strombus. He says : " (a) They may serve as nurse-cells to the eupyrene spermatozoa after copulation and before the latter reach the seminal receptacle ; (b) they may, by liberation of some substance,

stimulate the eupyrene spermatozoa or the eggs or both during fertilisation; or by the liberation of some substance to which the eupyrene spermatozoa are negatively chemotactic, they may act as an aid in the final disposition of the latter."

One remark naturally occurs on reading these suggestions It is that the vast majority of the Metazoa have no such noteworthy dimorphism in their spermatozoa as these Prosobranchs, and they overcome any possible difficulty in the nourishment, stimulation, and final disposition of their sperms without recourse to any atypic formations. Then why should certain molluscs depart from the usual methods in so far as to need a special new sort of sperm? Why should some other molluscs not have such special sorts of sperms? Experiments have shown that the apyrene spermatozoa degenerate sooner or later after undergoing katabolic changes in the albuminous bodies, that they are not so greatly stimulated by decaying tissue as are the eupyrene, and that the eupyrene spermatozoa live longer than the apyrene in sea-water (1). This all shows that the apyrene sperm lacks the energy of the eupyrene, and that it is in this way degenerate. For myself until further evidence of a more definite nature is brought forward I prefer to believe that these atypic spermatozoa are degeneration products produced by some altered condition in certain cells of the testis.

Whether these abnormal conditions are due to some alteration in the fluid nourishing and bathing the sperm-cells, and why these conditions should apply to some cells and not to others, are problems which in the case of the moths at least might be solved by further experiment, in the line already indicated. Finally, it should be remarked that Goldschmidt's hypothesis of altered conditions is to be regarded as more likely than any sex theory, for I feel convinced that in moths these spermatozoa arise out of a state of degeneration, and the varying conditions which are undoubtedly to be found during the metamorphosis of insects might possibly supply the disintegratory stimulus. It has already been pointed out where Goldschmidt's hypothesis seems to me to be weak.

## Addendum.

With regard to the matter of degeneration in the testes of moths, a very remarkable case has been brought to my notice by my friend, Dr. H. Eltringham, of New College, Oxford. A number of pupæ of the Emperor moth (Saturnia carpini) were purchased May, 1916, and none emerged that year. In 1917 about six moths emerged, paired, and laid fertile eggs. The rest of the pupæ failed to emerge. This June a number of the male gonads of these pupæ were sectioned, and it was found that they contained nothing except undeveloped spermatogonia and quite degenerate spermatocytes. There seems to be some correlation between the state of development of the gonads and time of emergence. The remaining pupæ do not appear to be going to emerge this year (July 29th, 1917).

## Summary.

(1) In Lepidoptera degeneration of cell elements take place at all stages of spermatogenesis.

(2) Degeneration of the chromosomes just after the second maturation division leads to what has been called "apyrene" spermatozoa.

(3) "Apyrene," "oligopyrene," and "eupyrene" spermatozoa are not separate kinds in Lepidoptera, but all intermediate stages are to be found.

(4) It is suggested that in Lepidoptera these terms lack the significance which has been attached to them.

(5) Degeneration may set in just when the cell elements are about to be properly orientated before spermiogenesis, and in such cases the nucleus and head centrosome fail to join. The former sinks down the lengthening sperm.

(6) The acroblasts almost always accompany the nucleus, and form a normal acrosome.

(7) In degenerate spermatids where the chromsomes fail to join up normally, the macromitosome (nebenkern) may be normally formed.

(8) The macromitosome may become normally elongated in sperms in which the nuclei are degenerate.

(9) It is suggested that the abnormal sperms are unable to bring about fertilisation.

(10) Individual nuclei can be reconstituted from separate chromosomes.

(11) At least partial inter-dependence of some cell elements is indicated by degenerate stages.

(12) Two centres of force, lodged respectively in the nucleus and in the centrosome, seem to be present.

DEPARTMENT OF PHYSIOLOGY, OXFORD ;
      March 19th, 1917.

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## EXPLANATION OF PLATE 26,

Illustrating Mr. J. Bronté Gatenby's paper, "The Degenerate (Apyrene) Sperm-formation of Moths as an Index to the Inter-relationship of the Various Bodies of the Spermatozoon."

### EXPLANATION OF LETTERING.

*A.* Acroblast or acrosome.   *C.* Centrosome.   *C.O.* Chromophobe vacuole of mitochondria.   *C.H.* Chromosomes.   *F.* Axial filaments. *F.W.* Follicle wall.   *G.* Granule.   *M.* Mitochondria (macromitosome). *M.I.* Micromitosome.   *N.* Nucleus.   *W.X.* Nucleus being formed from single chromosomes.   *X.Y.Z.* Different regions in the macromitosome.

[All figures drawn from an 18-compensating eyepiece and a Koritska $\frac{1}{15}$th semi-apochromatic oil immersion, with a camera lucida, at table level.   In reproduction figures reduced by one half.   Now × circa 2000 diameters.]

### PLATE 26.

Fig. 1.—Four spermatids of Smerinthus populi showing abnormal multinucleate condition resulting from a failure of the chromosomes to fuse up normally after second maturation division.   The mitochondria in the top left-hand cell are less abnormal than those of the other cells.

Fig. 2.—Spermatid of S. populi showing all cell elements normal except the nucleus, which has not been reconstructed from the chromosomes.   The latter, singly or in small numbers, are forming a group of separate nuclei.

Fig. 3.—Young spermatozoon of Pieris, quite normal except that the nucleus is falling away.   At *X.* is the front end of the macromitosomal spireme (January).

Fig. 4.—Second maturation division of S. populi, showing a lagging in the chromosomes.   The mitochondria are normal.

Fig. 5.—Advanced spermatozoon of Picris brassicæ, showing abnormal elongation of macromitosome.

Fig. 6.—Spermatid of Smerinthus populi with five nuclei; otherwise quite normal.

Figs. 7, 8, 10, and 11 show in P. brassicæ the falling away of the nucleus in the elongating spermatids.   Latter otherwise normal (January).

Fig. 9.—Oblique section of a sperm bundle of S. populi, showing normal acrosome formed near lagging nuclei, all of which have fallen away. Macromitosome normal.

Fig. 12.—Spermatozoon of S. populi, showing abnormal formation of macromitosome. From C. to Y. is normal. The part M. contains the main part of the mitochondrial matter, while at Z. there has been an attempt at elongation.

Fig. 13.—Spermatid with four nuclei, all of which stain as they do in normal stages. Cell otherwise normal, but each nucleus has a granule, which may or may not be derived from acrosomic matter. S. populi.

Fig. 14.—Spermatid of Pygæra bucephala, showing fairly normal macromitosome, but quite degenerate nucleus (October).

Fig. 15.—Mid part of bundle of metamorphosing spermatozoa of Pieris, showing abnormal acrosome not properly fused to the nucleus.